Supercritical CO_2 Extraction of Fat: Comparison of Gravimetric and GC–FAME Methods

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This study compares gravimetric and gas chromatographic (GC) fatty acid methyl ester (FAME) fat determinations of supercritical fluid extracts (SFE) from oilseeds, ground beef, bakery samples, and NIST Standard Reference Material 1544 (SRM-1544). SFE−GC−FAME and acid hydrolysis/solvent extraction (AH)/GC−FAME fat determinations are also compared. After extraction with supercritical CO_2 and ethanol, the collected material was weighed and total fat determined gravimetrically (SFE−GRAV). Subsequently, an internal standard was added and the material converted to FAMEs and analyzed by GC (SFE−GC−FAME). For sunflower and cottonseed, the SFE−GRAV results were higher than the SFE−GC−FAME results, whereas the two methods were equivalent for soybeans, canola, and safflower. For the ground beef samples, SRM-1544, and the emulsified "low-fat" bakery products, the SFE−GRAV results were significantly higher than both GC−FAME results (i.e, SFE and AH). For the "high-fat" bakery samples containing shortening, the SFE−GRAV, and GC−FAME results (i.e, SFE and AH) were in good agreement.

Keywords: *Supercritical fluid extraction; fat analysis; gas chromatography; fatty acid methyl ester; gravimetric*

INTRODUCTION

The determination of fat content is one of the most common analyses performed in a foodstuffs laboratory; however, quantitative extraction and analysis of fat are far from straightforward (Lumley and Colwell, 1991). Although the terms fat and lipid are often used interchangeably, they are not equivalent. The classical definition of lipid refers to any of various substances that are soluble in organic solvents including mono-, di-, and triglycerides, free fatty acids, phospholipids, sterols, lipoproteins, waxes, and hydrocarbons (Maxwell, 1987; Lumley and Colwell, 1991).

Although hexane is a good solvent for extracting free nonpolar lipids such as triglycerides, it is a poor solvent for polar lipids such as phospholipids and free fatty acids (Lumley and Colwell, 1991). In addition, the possible presence of "bound" lipids (Inkpen and Quackenbush, 1969) may require the use of polar solvents such as diethyl ether or chloroform/methanol to extract these compounds. However, these polar solvents tend to extract more nonfat compounds such as carbohydrates (e.g., sugars or starches), amino acids, peptides, and even water (Maxwell, 1987; Lumley and Colwell, 1991). Hydrolysis (acid, base, or enzyme) of the sample can also be used to release bound lipids; however, hydrolysis prior to solvent extraction tends to increase the amounts of nonfat extractables as well (McGhee et al., 1974).

Ideally, a fat extraction method would quantitatively remove all fat and only the fat from the matrix. This, however, is almost never the case, and it has been recognized for some time that some extracts do not contain pure triglycerides (Lepper and Waterman, 1925). Therefore, it is unlikely that a gravimetric measurement of the residue from a solvent extract of a sample, with or without prior hydrolysis, will give an accurate determination of fat content. In an effort to more accurately determine fat, the Nutrition and Labeling Education Act (NLEA) of 1990 defined fat as the sum of all fatty acids obtained from a total lipid extract expressed as triglycerides (*Federal Register*, 1993a). The NLEA protocol consists of the following steps: (1) a hydrolytic treatment; (2) solvent extraction of lipids; and (3) preparation of fatty acid methyl esters (FAMEs) for gas−liquid chromatography (GLC) and quantitation of saturated and unsaturated fat after stoichiometric conversion of FAMEs to triglycerides. To date, there are two methods approved by the Association of Official Analytical Chemists International (AOAC) using this definition of fat (House, 1997; Ngeh-Ngwainbi et al., 1997).

These methods, however, use large amounts of organic solvents (e.g., hexane, chloroform, or ether) in the tedious liquid−liquid extraction of fats. They also involve the time-consuming evaporation of the solvents prior to subsequent transesterification and analysis. Additionally, these solvents present potential hazards to both personnel and the environment. The use of organic solvents is coming under increased scrutiny, and the Environmental Protection Agency (EPA) has directed government agencies to reduce their consumption of solvents in federal laboratories (*Federal Register*, 1993b). Consequently, our research group has been investigating analytical supercritical fluid extraction (SFE) as an alternative to organic solvent-based extraction methods. SFE, especially supercritical CO_2 (SC-CO_2), is gaining acceptance due to its effectiveness, low toxicity, and reasonable cost. In addition, SC-CO_2 extraction methods can reduce extraction times, can be

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automated, and are environmentally safe. Eller and King's (1996) review of the SFE of foods for fat determination provides a list of foods extracted by SFE. Recently a collaborative study utilizing the SC-CO_2 extraction of fat from oilseeds (Am 3-96) has been approved (AOAC, −1997).

The extraction efficiency of SFE is generally compared to the extraction efficiency of solvent (Soxhlet or liquid−liquid) extractions by a gravimetric comparison of the weight of the whole extract and not a comparison of the specific components (e.g., triglycerides), although, recently, Taylor et al. (1998) made an indirect (i.e., separate extracts) comparison of SFE gravimetric and SFE−GC−FAME fat determinations. The purpose of this paper was to make a direct (i.e., same extract) comparison of the gravimetric determination and GC−FAME determination of SFE extracts for several different food matrixes and compare the SFE−GC−FAME method to a standard acid hydrolysis (AH) organic solvent extraction/GC−FAME method.

MATERIALS AND METHODS

Oilseed Samples. Five oilseeds (soybean, sunflower, canola, safflower, and cottonseed) were prepared by Mike Kennedy of Cargill Analytical Services (Minnetonka, MN) by milling to a fine powder and passing through a U.S.A. No. 20 sieve and were included in the 1995−1996 American Oil Chemists' Society (AOCS) Smalley Laboratory Proficiency Program (set I) (Taylor et al., 1998).

Ground Beef Samples. The three ground beef samples were prepared by the Department of Animal Science at the University of Illinois, Urbana, IL, and have been described by King et al. (1996).

Bakery Samples. The five bakery samples (bread with emulsifier, cake with emulsifier, cake with shortening, cookies with shortening/emulsifier, and crackers with shortening) were prepared by the American Institute of Baking (AIB) (Manhattan, KS) and are described by Ranhotra and Gelroth (1998). The emulsifier contained mono- and diglycerides, and the emulsifier-based products can be viewed as fatfree products. All samples were air-dried overnight at room temperature, finely ground, bagged, and frozen until being analyzed. The percent fat was determined for the "air-dried" samples and converted to "as-consumed" percent fat values using appropriate conversion factors (J. A. Gelroth, American Institute of Baking, Manhattan, KS, personal communication, 1997).

Standard Reference Material. Standard Reference Material 1544 (SRM-1544), "Fatty Acids and Cholesterol in a Frozen Diet Composite", was obtained from the National Institute of Standards and Technology (NIST) (Gaithersburg, MD).

Reagents. Fatty acid methyl ester (FAME) standards (i.e., GLC-85) and triundecanoin were purchased from Nu-Chek Prep (Elysian, MN). Boron trifluoride (14% BF_3 in methanol) was purchased from Alltech, Inc. (Deerfield, IL).

Supercritical Fluid Extraction (SFE). SFE was conducted with a Leco Corp. model FA-100 SFE (Leco Corp., St. Joseph, MI). Approximately 1 g of sample was weighed to the nearest 0.0001 g in a 50-mL beaker and mixed with ~1.5 g of Leco-Dry (Leco Corp.). This mixture was then added to the extraction thimble with a glass fiber filter disk (8 mm diameter, Leco Corp.) and ~0.5 g Leco-Dry on the bottom. Sufficient Leco-Dry was added to nearly fill the thimble, 1.0 mL of 100% ethanol (EtOH) was added, and a second glass fiber filter was placed on top. SFE was performed at 9000 psi and 100 °C at a flow rate of 2 L/min (measured at NSTP) for 25 min after an initial 5-min static hold. The variable restrictor was heated to 100 °C. Although Leco suggests that the collection vials be packed with glass wool, because of the difficulties in removing the collected fat from the glass wool, collection was performed in a 20-mL vial containing ~5 g glass helices (0.5 mm gauge, 4.2 mm diameter helix) to facilitate the subsequent transesterification. SFE/SFC grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) was used for all SFE experiments.

Fat Determination and Transesterification. The collected fat was weighed, and the gravimetric percentage fat (i.e., SFE−GRAV) was determined on the basis of the weight of original sample. Gas chromatography−FAME (GC−FAME) fat determinations were performed according to the general procedure described by House et al. (1994). One milliliter of a 10.00 mg/mL solution of triundecanoin in toluene was added to the collected fat residue on the glass helices along with 2 mL of 7% BF_3 in methanol. The vial was sealed with a Teflon-lined screw-cap and heated to 100 °C for 45 min with gentle mixing every 10 min. The vial was then allowed to cool to room temperature, and 5 mL of deionized water, 1 mL of hexane, and ~1 g of Na_2SO_4 were added and mixed vigorously. The vial was centrifuged to separate the layers, and the top layer was removed for subsequent GC−FAME analysis.

Acid Hydrolysis (AH) and Solvent Extraction of Bakery Samples and SRM-1544. Samples were digested by AH following the procedure of House et al. (1994). One milliliter of a 10.00 mg/mL solution of triundecanoin in chloroform was added to a 50-mL glass-stoppered Erlenmeyer flask and the solvent evaporated under a gentle stream of nitrogen. Approximately 1 g of sample was weighed to the nearest 0.0001 g into the flask, and ~100 mg of pyrogallol, 1 mL of EtOH, and 5 mL of 8.3 N HCl were added to the flask. The flask was stoppered and placed in a shaker bath set at 80 °C and 150 rpm. After 40 min, the flask was removed, allowed to cool to room temperature, and extracted with 25 mL of diethyl ether and 25 mL of hexane. The combined ether/hexane extracts were evaporated under nitrogen, and the residue was extracted with 5 mL of chloroform and transferred to a 12-mL screw-cap vial. The chloroform extract was evaporated under nitrogen, and the residue was transesterified (using toluene without triundecanoin) and analyzed by GC as described above.

GC Analysis and Quantification of Fat. Fat determination by GC−FAME analysis was performed according to the method of King et al. (1996). FAMEs were analyzed by split injection (200:1 split ratio) onto a Hewlett-Packard series II GC equipped with a flame ionization detector. The column used was an SP-2340 (60 m, 0.25-mm diameter, 0.20 μm film thickness) (Supelco, Bellefonte, PA) with He as the carrier gas at a linear flow velocity of 18 cm/s. The temperature program was 100 °C for 5 min, 3 °C/min to 190 °C, 1 °C/min to 200 °C, hold for 15 min, 50 °C/min to 250 °C, and hold for 1 min. The injector and detector temperatures were 235 and 250 °C, respectively. Injections were made using a Hewlett-Packard 7683 autoinjector, and the sample volume was 1 μL. The chromatographic data were acquired using a Hewlett-Packard Vectra VL2 computer and ChemStation software. The weights of the individual FAMEs were calculated on the basis of their integrations relative to the triundecanoin internal standard and were corrected using corresponding GC response factors for each fatty acid (House et al., 1994). The weights of the individual FAMEs were converted to equivalent weights of triglycerides using appropriate conversion factors (Carpenter et al., 1993). Total fat was calculated as the sum of all fatty acids expressed as triglycerides.

Statistical Analyses. Three replicate analyses were performed on each sample type−extraction method combination except for the bakery samples by AH−GC−FAME, for which there were five replicate analyses. Analyses of variance (ANOVA) were performed on percentage fat, after arcsin transformation (to stabilize variance) (Snedecor and Cochran, 1976), using Statistix 4.1 software (Analytical Software, Tallahassee, FL). Means were compared using linear contrast t tests or by least significant difference (LSD) at the $P = 0.05$ level.

RESULTS AND DISCUSSION

Oilseed Samples. The results of the total fat analyses of the five oilseed samples are shown in Table 1. Our SFE−GRAV determinations matched very well

Table 1. Mean[a] Percentage Total Fat (RSD) for Oilseed Samples

	SFE: CO_2/EtOH		SFE–GRAV[b]	
sample	gravimetric	GC–FAME	CO_2	CO_2/EtOH
soybean	20.0 (1.0) a	19.9 (0.8) a	19.2	20.5
sunflower	39.7 (1.7) a	38.8 (1.0) b	38.8	40.2
canola	39.3 (2.2) a	39.1 (2.1) a	37.7	40.2
safflower	36.3 (1.6) a	36.6 (1.9) a	35.7	37.1
cottonseed	19.2 (2.4) a	18.5 (1.7) b	19.1	19.7

[a] ($n = 3$), means within a row without letters in common differ significantly (linear contrast t test). [b] Data reprinted from Taylor et al. (1998).

Table 2. Mean[a] Percentage Total Fat (RSD) for Ground Beef Samples

nominal	SFE: CO_2/EtOH		AH/solvent
% fat	gravimetric	GC–FAME	extraction/GC–FAME[b]
10	13.3 (6.8) a	12.0 (4.3) b	12.8 (5.6) ab
20	23.3 (5.8) a	22.5 (1.4) ab	21.8 (4.5) b
30	29.5 (1.4) a	28.8 (1.1) a	28.6 (3.7) a

[a] ($n = 3$), means within a row without letters in common differ significantly (linear contrast t test). [b] AH–GC–FAME analyses performed by Medallion Labs, Minneapolis, MN, taken from King et al. (1996).

with those reported previously (AOCS/AOAC Collaborative Study, set I) for these same oilseeds with our values between the neat CO_2 and the CO_2/EtOH values (Taylor et al., 1998). This result is probably due to our "one-time" static addition of 1 mL of ethanol being intermediate in extraction efficiency between the neat CO_2 method and the CO_2/EtOH method, which maintained a constant percent of EtOH (i.e., 15%) over the entire extraction period (Taylor et al., 1998). As expected, the ANOVA revealed there were significant differences ($F_{4,10} = 20808$, $P < 0.001$) in total fat content among the five oilseeds examined. The SFE–GRAV and SFE–GC–FAME results were similar, although the SFE–GRAV results were, in general, slightly higher than the SFE–GC–FAME results. The ANOVA revealed a significant main effect of fat determination method ($F_{1,10} = 22.5$, $P < 0.001$), and the overall SFE–GRAV mean percent fat (30.89) was significantly higher than the overall SFE–GC–FAME mean percent fat (30.59). The ANOVA also revealed a significant oilseed type by fat determination method interaction ($F_{4,10} = 11.3$, $P < 0.001$). The linear contrasts comparing the two methods for each of the five oilseeds indicated that the two methods were significantly different only for sunflower ($T = 6.23$, $P < 0.001$) and cottonseed ($T = 4.84$, $P < 0.001$). For soybeans, canola, and safflower, the two methods were statistically equivalent ($T = 0.69$, $P = 0.50$; $T = 0.92$, $P = 0.38$; $T = 2.08$, $P = 0.065$, respectively). Apparently, for soybeans, canola, and safflower, there is essentially quantitative extraction of the fat without extraneous nonfat material. However, for sunflower and cottonseed, there is some nonfat material extracted with the CO_2/EtOH used to extract the oilseeds.

Ground Beef Samples. The results of the total fat analyses for the three ground beef samples are shown in Table 2. Our SFE–GC–FAME determinations matched very well with the AH–GC–FAME results reported previously for these ground beef samples (King et al., 1996). The ANOVA revealed there were significant differences ($F_{2,18} = 929$, $P < 0.001$) in total fat content between the three ground beef samples (i.e., ca. 10, 20, and 30% fat) examined. There was not a significant ground beef sample by fat determination method interaction ($F_{4,18} = 0.68$, $P = 0.62$). The ANOVA did reveal a significant main effect of fat determination method ($F_{2,18} = 4.13$, $P = 0.033$). The overall SFE–GRAV mean percent fat (22.03) was significantly higher than both the overall SFE–GC–FAME mean percent fat (21.10) and the overall AH–GC–FAME mean percent fat (21.08). This indicates that there was some nonfat material extracted along with the fat by the SFE method. King (1994) previously reported that gravimetric fat determinations for ground beef samples were ~0.5% higher than the GC–FAME determinations. It is interesting to note that for the ground beef samples, the difference between the SFE–GRAV determination and the SFE–GC–FAME determination decreased with increased fat content of the ground beef samples (i.e., the extraneous material extracted was inversely proportional to the fat content). Although the nature of the extraneous material is not known, it is possible that decreased amounts of fat allow increased amounts of water to be extracted. Water is somewhat soluble in SC-CO_2 (Evelein et al., 1976).

Table 3. Mean[a] Percentage Total Fat (RSD) for Bakery Samples

	SFE: CO_2/EtOH		AH/ solvent extraction/
sample	gravimetric	GC–FAME	GC–FAME[b]
bread with emulsifier	0.91 (2.8) a	0.54 (2.1) b,2	1.03 (11.5) 1
cake with emulsifier	0.40 (16.9) a	0.32 (12.0) b,1	0.32 (7.0) 1
cake with shortening	11.8 (3.1) a	11.8 (3.2) a,1	11.9 (2.1) 1
cookies with emulsifier/ shortening	20.7 (2.6) a	20.7 (2.8) a,1	20.9 (2.0) 1
crackers with shortening	10.2 (1.8) a	10.1 (1.5) b,2	10.4 (1.5) 1

[a] Means within a row without letters (SFE gravimetric versus SFE–GC–FAME values) or numbers (SFE–GC–FAME versus AHSE–GC–FAME values) in common differ significantly (linear contrast t test). [b] Ranhotra et al. (1998) reported AHSE–GC–FAME values of 1.37, 0.51, 12.2, 21.1, and 10.6, respectively, for these samples.

The overall SFE–GC–FAME and AH–GC–FAME means were statistically equivalent. This indicates that the SFE–GC–FAME method quantitatively removed and accurately determined the fat from the ground beef samples. Even though the SFE method may have extracted some nonfat material from the ground beef, the extraneous material was excluded during the GC–FAME analysis.

Bakery Samples. The results of the total fat analyses for the five bakery samples are shown in Table 3. In general, our AH–GC–FAME determinations were very close to the AH–GC–FAME values previously reported, although our values were on average ~0.5% lower than those reported earlier (Ranhotra and Gelroth, 1998). It is possible that these discrepancies may, in part, be explained by our incomplete extraction due to the fact we used only 50 mL of ether/hexane in our extractions and Ranhotra and Gelroth (1998) used 110 mL.

The ANOVA comparing the SFE–GRAV results with the SFE–GC–FAME results revealed there was a highly significant main effect of bakery product ($F_{4,10} = 317276$, $P < 0.001$) as well as a significant bakery product by fat determination method interaction ($F_{4,10} = 25.2$, $P < 0.001$) on total fat content. The ANOVA also revealed a significant main effect of fat determination method ($F_{1,10} = 74.2$, $P < 0.001$). The overall

Table 4. Mean[a] Percentage Total Fat (RSD) for NIST SRM-1544[b]

extraction/analytical method	mean percentage total fat (RSD)
SFE: (CO_2/EtOH) gravimetric	4.61 (8.29) a
SFE: (CO_2/EtOH) GC–FAME	3.52 (4.59) b
AH/solvent extraction/GC–FAME	3.60 (0.51) b

[a] (n = 3), means without letters in common differ significantly (least significant difference). [b]NIST noncertified total fat = 3.68%.

SFE–GRAV mean percent fat (8.80) was significantly higher than the overall SFE–GC–FAME mean percent fat (8.69). The linear contrasts comparing the two methods for each of the five bakery products indicated that the SFE–GRAV and SFE–GC–FAME methods gave significantly different results for bread with emulsifier (T = 12.3, P < 0.001) and cake with emulsifier (T = 2.80, P = 0.019). Although the linear contrast comparing the SFE–GRAV and SFE–GC–FAME methods for crackers with shortening was also statistically significant (T = 3.47, P = 0.006), the actual difference between the two methods for this sample (i.e., 0.1%) was negligible. For these three types of bakery samples, the CO_2/EtOH apparently extracted extraneous nonfat material. However, for cake with shortening and cookies with shortening/emulsifier, the SFE–GRAV and SFE–GC–FAME methods were statistically equivalent (T = 0.97, P = 0.35; and T = 1.65, P = 0.13, respectively). For these two bakery samples, essentially everything extracted was fat.

The ANOVA comparing the SFE–GC–FAME results with the AH–GC–FAME results indicated that the two fat determination methods were significantly different for bread with emulsifier (T = 6.85, P < 0.001) and crackers with shortening (T = 2.95, P = 0.026). For these two bakery samples, the treatment by acid hydrolysis apparently releases some bound fat not otherwise available for extraction by SFE. However, the SFE–GC–FAME and AH–GC–FAME methods were statistically equivalent for cake with emulsifier (T = 0.11, P = 0.91), cake with shortening (T = 0.30, P = 0.78), and cookies with shortening/emulsifier (T = 0.64, P = 0.55).

It is interesting to note that for the cake with emulsifier sample, although the SFE–GRAV method apparently extracted material other than fat, the fat in this sample was quantitatively extracted by the CO_2/EtOH and the SFE–GC–FAME result was equivalent to that of the AH–GC–FAME method. For the cake with emulsifier sample, if one only compared the SFE–GRAV results to the AH–GC–FAME results, one might conclude that the SFE method was more effective in extracting fat than the AH method, when, in fact, it was less effective in extracting fat. Conversely, for bread with emulsifier, the SFE–GRAV method extracted extraneous nonfat material but did not quantitatively remove all of the fat, giving falsely close agreement to the AH–GC–FAME result.

Standard Reference Material. The results of the total fat analyses for SRM-1554 are shown in Table 4. The ANOVA revealed a significant main effect of fat determination method ($F_{2,8}$ = 19.2, P = 0.003) on percent total fat. The SFE–GRAV mean percent fat (4.61) was significantly higher than both the SFE–GC–FAME (3.52) and AH–GC–FAME (3.60) means. However, the SFE–GC–FAME and AH–GC–FAME methods were statistically equivalent, and these two methods gave results very close to the noncertified value reported by NIST (i.e., 3.68% fat). Apparently, the CO_2/EtOH SFE method did extract some nonfat material from this matrix, although it did quantitatively extract all of the fat.

Table 5. Mean[a] (RSD) Percentage Individual Fatty Acids in NIST SRM-1544

fatty acid	SFE: CO_2/EtOH/ GC–FAME	AH/solvent extraction/ GC–FAME	NIST value
lauric	0.152 (4.12) a	0.158 (0.63) a	0.131[b]
myristic	0.109 (4.51) a	0.113 (1.34) a	0.101[b]
palmitic	0.647 (4.48) a	0.714 (0.29) b	0.577[b]
stearic	0.226 (4.88) a	0.227 (0.25) a	0.200[b]
oleic	1.344 (4.66) a	1.380 (1.27) a	1.164[b]
linoleic	0.715 (4.34) a	0.737 (0.21) a	0.656[b]
caprylic	0.031 (3.77) a	0.032 (1.79) a	0.03[c]
capric	0.032 (3.65) a	0.033 (0) a	0.03[c]
palmitoleic	0.036 (4.81) a	0.038 (0) a	0.04[c]
linolenic	0.070 (7.01) b	0.060 (1.67) a	0.06[c]

[a] (n = 3), means within a row without letters in common differ significantly (linear contrast t test). [b] NIST certified value. [c] NIST information value.

The amounts of individual fatty acids found in SRM-1544 by the SFE–GC–FAME and AH–GC–FAME methods are shown in Table 5. In general, the amounts of the individual fatty acids as determined by these two methods agreed very well with the values given by NIST for this material. Although the two extraction methods yielded statistically equivalent amounts for 8 of 10 of the individual fatty acids, the AH–GC–FAME method yielded significantly more palmitic acid (T = 4.05, P = 0.015) and significantly less linolenic acid (T = 3.72, P = 0.021) than did the SFE–GC–FAME method. It is possible that the AH method released some palmitic acid that may have been bound and not extracted by the SFE method. The decreased amount of linolenic (although very slight) was probably a result of the degradation of this fatty acid by the AH treatment. Linolenic acid is the most labile of the fatty acids determined.

The relative standard deviations (RSDs) (or coefficients of variance) for the SFE–GRAV analyses were greater than those for the SFE–GC–FAME analyses in 11 of 14 cases (Tables 1–4). The probability of obtaining this result, assuming the RSDs of the two analytical methods are equivalent, is P = 0.029 (binomial probability distribution). Therefore, we conclude that the variability for the SFE–GC–FAME method is less than that for the SFE–GRAV method and that the higher variability is due to a higher variability in the extraction of nonfat material and not to variability in the extraction of fat. The GC–FAME analysis seems to "correct" for this variability because it is specific for fatty acids and extraneous nonfat material is excluded.

Assuming that AOAC-approved AH–GC–FAME methods give the most accurate determination of fat (House, 1997; Ngeh-Nwainbi et al., 1997), other methods which match or exceed the AH–GC–FAME results are, at first glance, apparently as effective or more effective methods. However, occasionally SFE–GRAV and AH–GC–FAME determinations were in close agreement, with the SFE–GC–FAME determination significantly less than the SFE–GRAV value (e.g, bread with emulsifier, cake with emulsifier, and crackers with shortening). This suggests that the apparent agreement between the SFE–GRAV and the AH–GC–FAME results was fortuitous and, undoubtedly, a result of extraction of nonfat material coupled with incomplete extraction of fat.

The results of our direct comparison of gravimetric and GC–FAME fat determinations for several matrixes indicated that the two methods were equivalent for 7 of 14 cases (i.e., soybeans, canola, safflower, 20 and 30% ground beef, cake with shortening, and cookies with emulsifier/shortening). In these examples, the SFE–GRAV method gives an accurate as well as easy and fast determination of the fat content. For these matrixes, the SFE method we used is an excellent alternative to solvent-based methods in quality control fat determination applications. In the remaining 7 cases, the gravimetric method values were significantly higher than the GC–FAME method values. For "wet" samples (i.e., ground beef and SRM-1544) the high gravimetric values are probably due to extracted water, and it may be possible to bring the gravimetric and GC–FAME into agreement by eliminating water from the extract either before the extraction (e.g., by lyophilization), during the extraction (e.g., by the addition of a drying agent in the extraction cell), or after the extraction (e.g., by a vacuum oven). For the "dry" samples, the identity of the extraneous material is less clear and a means of eliminating these compounds more difficult to envision.

The results of the comparisons of the SFE–GC–FAME and AH–GC–FAME fat determination methods were equivalent in seven of nine cases (i.e., all three ground beef samples, cake with emulsifier, cake with shortening, cookies with emulsifier/shortening, and SRM-1544), and the SFE–GC–FAME method gives both a specific fatty acid content and an accurate total fat determination. For these seven matrixes, the SFE method is again an excellent alternative to solvent-based methods for NLEA fat determination applications. For the remaining two cases (i.e., bread with emulsifier and crackers with shortening), the SFE method gave significantly lower results than did the AH method. This difference is undoubtedly a result of the SFE method not extracting bound lipids, and it may be possible to bring these two methods into agreement by the integration of a hydrolysis step into the SFE method.

In conclusion, SFE is demonstrated to be a suitable replacement for traditional organic solvent extraction for the determination of fat content in some food products, and with continued method development an increasing number of foods can be analyzed by using this technique. SFE methodology also eliminates the tedious and time-consuming liquid–liquid extraction and solvent evaporation steps, along with the attendant risks associated with personnel exposure to toxic solvents, use of hazardous chemicals, and solvent disposal problems.

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